Tetrahedron 72 (2016) 605–612

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Approaches to polyunsaturated sphingolipids: new conformationally restrained analogs with minimal structural modifications

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ARTICLE INFO

Article history:
Received 15 October 2015
Received in revised form 27 November 2015
Accepted 30 November 2015
Available online 15 December 2015

Keywords:
Sphingolipids
Diastereoselectivity
Polyene
Hydrozirconation
Ceramide
Sphingosine

ABSTRACT

Conformationally restrained sphingolipids **1**–**4**, arising from the introduction of a polyene or a polyenyne moiety as part of the sphingoid backbone, have been synthesized. While addition of polyene acetylides **6** and **7** to Garner's aldehyde afforded the corresponding Felkin-Anh *anti*-addition adducts, addition of alkenylzirconocenes showed no diastereoselectivity. This behaviour was also observed from protected serinal **14**, which allowed the synthesis of the acid-sensitive pentaene sphingosine backbone present in ceramide **4**. Despite the inherent limitations of the synthetic approaches here reported, their application to such highly conjugated polyene sphingoid analogs is unprecedented.

1. Introduction

One of the ultimate goals in biology is to understand the relationship between structure, function, and dynamics of biomolecules in their natural environment, namely the living cell. In this context, the use of conformationally restrained analogs has become a common strategy in medicinal chemistry programs to delineate the active conformation of flexible molecules and, indirectly, to gain insight into the three-dimensional requirements of their receptors.[1] This approach has also been applied in the sphingolipid arena by incorporating part of the aminodiol moiety of the sphingoid backbone into cyclic structures[2–4] or, less frequently, by introduction of unsaturations in the sphingoid chain.[5–10] However, the full understanding of the roles of sphingolipids (SLs) in the cell environment is far for complete due to a lack of adequate experiments able to directly visualize molecular interactions under physiological conditions. In this context, fluorescent lipid analogs become useful biophysical tools. In particular, polyene systems[11–14] are suitable fluorescent tags that introduce minimize structural alterations in the resulting probe,[15] in comparison with commercially available bulkier chromophores, such as BODIPY or NBD.[16,17] Most importantly, the pentaene moiety has been reported to behave like its endogenous saturated counterparts in vivo, with respect to uptake, metabolism, transport and localization in membrane microdomains.[12,18,19] However, when these labeled lipids are part of the *N*-acyl or *O*-acyl SLs chains,[11,12,15,20,21] the fluorophore can be released by hydrolysis of the amide or the ester bonds by specific enzymes, and the resulting sphingoid base is no longer traceable. This is not the case when the polyene system is part of the sphingoid backbone. On the other hand, the chemical modification of the sphingoid backbone by incorporation of an acetylene group has received considerable attention.[8,22–24] The triple bond increases the rigidity of the molecule, affecting its physicochemical and biological properties.

Based on the above considerations and in our interested in the development of new sphingolipid probes with potential biophysical and biochemical applications, we wish to report on the development of synthetic protocols for the elaboration of new conformationally constrained sphingosines and ceramide analogs resulting from the incorporation of a polyunsaturated system as part of the sphingoid backbone. In this account, the synthesis of

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http://dx.doi.org/10.1016/j.tet.2015.11.067
0040-4020/

polyenyne sphingosines **1** and **2**, polyene sphingosine **3** and polyene ceramide **4** are described as representative examples of the above general approach (Fig. 1).

2. Results and discussion

Access to the alkyne polyene framework present in probes **1** and **2** was planned by nucleophilic addition of the corresponding lithium acetylides of **6** or **7** (Scheme 1) to Garner's aldehyde under conditions similar to those described for simpler acetylenes.[23,25–27] The synthesis of the required acetylenes started from the phosphonium salt **5**, easily obtained in two steps (85% overall yield) from commercially available 4-pentyn-1-ol.[28] Salt **5** was submitted to (*E*)-selective Wittig olefination[29] with the required polyene aldehyde (2,4-hexadienal for **6** and 2,4,6,8-decatetraenal[30] for **7**), followed by iodine-promoted isomerization[31–34] of the resulting polyene system. The initial condensation attempts of **6** with Garner's aldehyde, using BuLi/HMPA or LiHMDS in THF at low temperature for acetylide generation, were unsuccessful. Only the use of LDA in THF at −78 °C afforded acceptable yields of the condensation adduct **8**. Application of the above conditions to acetylene **7** led to the corresponding adduct **9**, albeit in lower yields. Gratifyingly, only the corresponding C3-*erythro* isomers of **8** and **9** were obtained, in agreement with the operation of a Felkin-Anh transition state model. The low temperature used in this condensation step becomes crucial for the exclusive formation of the *erythro* isomers and avoids the use of HMPA or HMPT as co-solvents.[23] In our case, the *erythro* configuration of **8** and **9** was confirmed by comparison with the reported ^{1}H NMR data for the corresponding saturated alkyne in $CDCl_3$.[35] The simultaneous removal of the isopropylidene and Boc groups of the above adducts under acidic conditions (HCl in MeOH)[36] led to the target acetylene polyene probes **1** and **2** in acceptable yields (Scheme 1). Contrary to our initial expectations, the acidic conditions used in this deprotection step were compatible with the presumably acid-sensitive polyene framework of the sphingoid backbone.

For the synthesis of the polyene probes **3** and **4** (Fig. 1), initial attempts relied on the stereoselective Red-Al® reduction of the propargylic alcohols **8** and **9** to the corresponding *E*-allylic alcohols, following the reported methodology of Herold et al.[37] However, complex reaction mixtures were obtained in both cases, as well as from the fully deprotected amino diol **1**. In light of these results, hydrozirconation of the alkyne polyenes **6** and **7** with Schwartz reagent ($Cp_2Zr(H)Cl$),[38] followed by addition of the intermediate alkenyl zirconocenes to Garner's aldehyde, was considered (Scheme 2). According to the literature, this sequence has been successfully applied to the elaboration of the allylic alcohol framework present in simple sphingolipids and related compounds.[39–44] In particular, the addition of alkenyl zirconocenes to Garner's aldehyde has been reported to lead to *anti* adducts by the use of substoichiometric $ZnBr_2$ as carbonyl-activating Lewis acid. On the other hand, by addition of a transient alkenylzinc intermediate, arising from transmetallation of the above alkenyl zirconocene, the opposite *syn* stereoselectivity is obtained.[41,42] In our case, initial hydrozirconation attempts of **6** using $ZnBr_2$ as Lewis acid were unsuccessful, since no addition products were obtained. After much experimentation (variation of $ZnBr_2$ stoichiometry, solvent systems,[45] and even the in situ generation of Schwartz reagent[46]) we turned our attention to the use of silver salts, which have also been reported as suitable catalysts for the addition of alkenyl zirconocenes to aldehydes.[47–49] Thus, coupling of Garner's aldehyde, in the presence of $AgClO_4$ (20% mol), with the alkenyl zirconocene arising from alkyne **6** (5*E*/*Z*: 4/1) afforded the desired addition product **10** in 50% yield in a roughly 1:1 *syn*/*anti* ratio, as expected from the reported drop of diastereoselectivity under silver promoted additions.[41,47] Similarly, the hydrozirconation-coupling of alkyne (all-*E*)-**7** with Garner's aldehyde under the above conditions led to the expected adduct **11** (*syn*/*anti* 1:1), albeit in lower yield (Scheme 2). Despite the simultaneous deprotection of the isopropylidene and *N*-Boc groups of **10** in HCl–MeOH afforded the expected polyene sphingosine **3**, these conditions were not suitable for the deprotection of pentaene **11**, since a complex mixture of products were obtained in all cases.

In light of the above results and taking into account the apparent instability of the conjugated pentaene system under the acidic conditions required for the deprotection step, we considered the use of an alternative protection strategy in the starting aldehyde. Thus, the previously unreported protected serinal **14** was considered for our purposes. In this case, the Fmoc and TBDMS protecting groups would enable the use of non-acidic deprotection conditions in the final step. The protected serinal **14** was obtained from Fmoc serine methyl ester, after hydroxyl protection (as the TBDMS ether **12**) and reduction of the intermediate Weinreb amide **13** (Scheme 3). Alkenylation of aldehyde **14** was optimized using 1-tetradecenylzirconocene as a model, obtained from treatment of 1-tetradecyne with Schwartz reagent. Under the above silver promoted alkenylation conditions, a marked stereoselectivity leading to the unnatural *syn* adduct (*anti*/*syn* 1:4) was observed. The configurational assignment at C3 in this mixture was unambiguously confirmed by its derivatization to the corresponding (*R*) and (*S*)-MPA esters, following the standard methodology of Riguera et al.,[50] as illustrated in Fig. 2.

Attempts to revert this stereoselectivity were next undertaken. After much experimentation, the use of $ZnCl_2$ (50 mol %) in DCM allowed the alkenylation of **14** with polyene (all-*E*)-**7** to afford the adduct **15** as an *anti*/*syn* 1:1.5 mixture, albeit in modest yield (Scheme 3). The diastereoisomeric composition of **15** was inferred from that of the above model reaction of aldehyde **14** with 1-tetradecyne.

In order to avoid an excessive manipulation of the sensitive polyene sphingosine system, we decided to carry out an one-pot deprotection-acylation of **15**. Thus, the simultaneous removal of

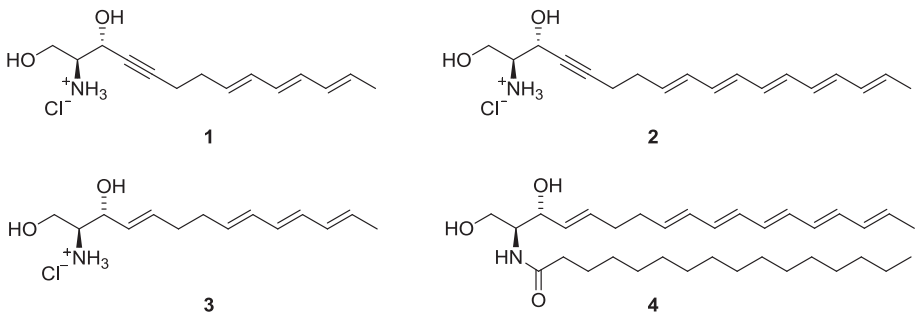

Fig. 1. Unsaturated probes described in this work.